

The University of Chicago

RÔLE OF OMENTUM OF RABBITS,
DOGS AND GUINEA-PIGS IN
ANTIBODY PRODUCTION

A DISSERTATION

SUBMITTED TO THE FACULTY
OF THE OGDEN GRADUATE SCHOOL OF SCIENCE
IN CANDIDACY FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

DEPARTMENT OF PATHOLOGY

BY

BERNARD PORTIS

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RÔLE OF OMENTUM OF RABBITS, DOGS AND GUINEA-PIGS IN ANTIBODY PRODUCTION

BERNARD PORTIS

From the Department of Pathology of the University of Chicago and from the Snyder Fund of the Michael Reese Hospital (Nelson Morris Institute for Medical Research)

It has been determined that certain organs and tissues are involved in the production of antibodies in response to intraperitoneal injection of various antigens. The main methods of attack have been the removal of organs and the inhibition or destruction of their function through the use of different physical and chemical agents, before and after the introduction of the antigenic substances.

The omentum has great phagocytic power, but it remains to determine whether it also takes part with the spleen, bone marrow, and other organs in the development of antibodies. Because of its accessibility and because it permits morphologic study of the interaction between its different cell types and intraperitoneally introduced antigens, the omentum offers a field for the examination of such relations as may exist, if any, between a local defensive reaction such as expressed by phagocytosis and a more general protective reaction evidenced by the formation of diffusible antibodies.

LITERATURE

FUNCTIONS OF THE OMENTUM

The omentum plays such a definite rôle in both normal and pathologic abdominal conditions that its importance should not be lost sight of, as a brief résumé of its various functions will clearly show.

Absorption.—That fluids are absorbed from the peritoneal cavity was known by Aristotle. The route of absorption is still in dispute. Shippley and Cunningham¹ demonstrated that "true and pseudo-solutions, and granules of particulate matter" are absorbed by the omental vessels. The lymphatic plexuses on the under surface of the diaphragm may also absorb considerable amounts of fluid and soluble elements (Bolton,² Buxton,³ and Wells⁴). Ziegler⁵ found that small and even relatively large foreign bodies were absorbed from the peritoneal cavity, mainly through the large network of subendothelial cells and not

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¹ Am. Jour. Physiol., 1915, 40, p. 75.

² Jour. Path. and Bacteriol., 1921, 24, p. 429.

³ Jour. Med. Res., 1906, 15, p. 189; 1907, 16, p. 17.

⁴ Jour. Infect. Dis., 1907, 4, p. 582; Am. Jour. Physiol., 1907, 18, p. 156.

⁵ Ztschr. f. d. ges. exper. Med., 1921, 24, p. 223.

through preformed spaces. Wilkie⁶ determined that more saline solution was absorbed in animals with the omentum than those deprived of this structure, in a ratio of 3 to 2. This result was confirmed by Rubin.⁷ Morrison⁸ likewise observed that the absorption of lymph was facilitated by increased vascularity of the omentum. Clark⁹ states that the differences in the rate of absorption of various fluids from the peritoneal cavity are dependent on the variations in osmotic pressure. Posture seems to have little effect on the rapidity of absorption (Rowntree and Dandy¹⁰).

Reaction to Foreign Particles Introduced in the Peritoneal Cavity.—That the omentum can engulf foreign bodies introduced into the peritoneal cavity has been clearly demonstrated by Wilkie,⁶ Durham,¹¹ Hertzler,¹² LePlay,¹³ and many others. It has also been shown that animals deprived of the omentum frequently succumb to injections of charcoal. LePlay¹³ found that even the resected omentum, when left in the peritoneal cavity, retained the power to react to foreign bodies.

The Movement of the Omentum to Infected Areas, and Its Plastic Effects.—It is well known that the omentum, in a considerable number of cases, seems to move to the site of acute peritoneal inflammation. There is still considerable difference in opinion as to the apparent process of motion. The omentum, because of its protective rôle, has been called "abdominal policeman," "friend in need," "the great leukocyte." Norris¹⁴ showed that it is entirely devoid of muscle, and possesses no inherent power of movement. It seems most logical to accept the view of Rubin,⁷ Fisher,¹⁵ Bloomhardt, Andrew and Hetherington,¹⁶ and Wilkie,⁶ that in the presence of a localized peritoneal infection, active peristalsis of the intestine pushes the omentum toward the inflamed peritoneum. However, Morrison,⁸ Aimes,¹⁷ and others maintain, chiefly from clinical evidence, that the omentum changes its position with a sort of intelligence, not due primarily to mechanical causes. Morrison⁸ believes that the motion is very rapid, while Wilkie⁶ considers it to be a matter of hours or days before it is successfully completed.

The plastic effect has been frequently demonstrated by general surgeons, and experimentally by Bloomhardt, Andrews and Hetherington.¹⁶ These authors found that the omentum was capable of closing experimental perforations. Many older surgeons—Senn, Mayo, and others—have shown the great clinical value of using the omentum to repair defects in the gastro-intestinal tract, and sealing tight the line of suture in intestinal anastomosis.

The Bactericidal Function of the Omentum.—The omentum contains many phagocytic cells (Metchnikoff), singly and in islands along the blood vessels, and Wilkie⁶ secured positive bacterial cultures from the omentum, when the exudate had become sterile after injection of broth cultures into the peritoneal cavity. Norris¹⁴ believes that the main function of the omentum is that of leukocyto-genesis. Buchtel¹⁸ found that animals deprived of the omentum were less resistant

⁶ Brit. Med. Jour., 1911, 2, p. 1103.

⁷ Surg., Gynec. & Obst., 1911, 12, p. 117.

⁸ Brit. Med. Jour., 1906, 1, p. 76.

⁹ Jour. Pharm. & Exper. Therap., 1921, 16, p. 415.

¹⁰ Ann. Surg., 1914, 59, p. 44.

¹¹ Jour. Path. & Bacteriol., 1892, 4, p. 338.

¹² The Peritoneum, 1919.

¹³ Compt. rend. Soc. de biol., 1911, 71, p. 484.

¹⁴ Univ. Pennsylvania Med. Bull., 1908, 21, p. 119.

¹⁵ Brit. Med. Jour., 1906, 2, p. 325.

¹⁶ Surg., Gynec. & Obst., 1917, 34, p. 474.

¹⁷ Progrès méd., 1920, 35, p. 8, 12.

¹⁸ Denver Med. Times and Utah Med. Jour., 1911, 31, p. 305.

than normal animals to induced peritoneal infection. Dudgeon and Ross¹⁹ have demonstrated that the omentum becomes intensely injected in experimental peritonitis, and also confirmed the findings of Wilkie. Rubin⁷ likewise agrees with the other authors in the great phagocytic power of the omentum.

Vascularization of Tumors and Reaction to Strangulated Organs.—De Renzi and Boeri²⁰ have found that when the blood supply of the spleen or kidney was ligatured, the omentum usually encapsulated the infarcted organ and the animals survived, whereas when the omentum was previously removed, they invariably died. Furthermore, they determined that the omentum could supplement a partial interference with the arterial supply of the spleen. The spleen, in the animals which survived the complete occlusion of vascular supply, was completely encapsulated in the omentum. This function has been further studied in experimental strangulation of large segments of the gastro-intestinal tract (Bloomhardt, Andrews and Hetherington¹⁶ and others). The vascularization of abdominal tumors through omental adhesions has been seen by all surgeons and pathologists. The readiness with which a rich vascular supply may be developed through omental adhesions is utilized in the various operations of omentopexy for cirrhosis of the liver.

Summary.—The omentum is an important factor in absorption; it is capable of walling off localized peritoneal infections, and of encapsulating foreign bodies; it has considerable anti-infectious power; and it may be used to furnish a new vascular supply to strangulated organs.

THE STRUCTURE OF THE OMENTUM

The omentum of many different species has been studied carefully. The rabbit omentum, however, has been the one of choice for experimental work. Norris,¹⁴ in a complete review of this structure, described it accurately:

The omentum consists of a serous membrane, which hangs, apron like, from its attachments to the stomach, transverse colon, gastrohepatic and gastrosplenic ligaments. It consists of 4 layers of peritoneum between which are enclosed arteries, veins, lymphatics, nerves, fat, and fibrous tissue, also many cellular accumulations. The arterial supply is derived from the gastro-epiploic, middle colic, splenic, and duodenal arteries. These vessels send off numerous treelike branches, usually at right angles to the main trunks. There are, however, many avascular areas. The cellular aggregations are chiefly found along the vessels. The lymphatics form a well defined system, the larger vessels of which follow the larger arteries, and have the usual structure, consisting of an intima, media and adventitia. The smaller lymphatics consist of a single layer of endothelium. Norris believes that there are definite stomata, but later work seems to have disproved this. Hertzler¹² states that the lymphatics are poorly defined and few in number.

Cunningham²¹ considered the rabbit omentum a characteristic structure, consisting of a framework of blood vessels which anastomose freely without the

¹⁹ Am. Jour. Med. Sc., 1906, 132, p. 37.

²⁰ Berl. klin. Wehnschr., 1903, 34, p. 773.

²¹ Bull. Johns Hopkins Hosp., 1922, 33, p. 257.

intervention of an elaborate capillary bed and that much of the circulation is arteriovenous in character. The larger vessels are surrounded by layers of adipose tissue. The thin membranous part consists of numerous cells interspersed in a mass of interlacing connective tissue fibrils and covered by a layer of flattened mesothelial cells, whose outlines can be demonstrated by the mosaic pattern which they evidence after treatment with silver nitrate. The special structures which are so evident in the rabbit omentum are the "taches laiteuses" scattered more or less indiscriminately throughout the omentum. These "taches laiteuses" or milky spots are formed by collections of various types of free cells grouped about areas of increased vascularity. These cellular islands were first described by Ranvier, so that they are frequently called "taches laiteuses de Ranvier." Weidenreich²² believed that the omentum was wholly analogous to a lymph gland, the mesh work corresponding to the reticulum of the gland and the free cells and the "taches laiteuses" to the pulp cells and the follicles, respectively.

The histology of the omentum has afforded an interesting field for research, because of its varied cellular content. Cunningham²¹ has recently stated that the work along this line could be divided into two great groups: one including the genetic classification of the cells which constitute the "taches laiteuses" of the rabbit omentum; the other concerning the characteristics and relationship between the three great groups of cells which constitute the framework of the organ—the clasmatocytes, fibroblasts, and the cells of the serosal lining.

The relation of the serosal cells to the fibroblasts has aroused considerable discussion. Ranvier,²³ in 1891, noticed that after setting up a peritoneal irritation with silver nitrate the superficial cells were shed and seemed to be replaced by the deeper cells, and hence concluded that the two types of cells were indistinguishable and interchangeable. Dominici,²⁴ in 1901, considered the component cells of the omentum as representing various cell syncytiums which were not entirely separated but belonged collectively to the connective tissue series and were interchangeable. Schott²⁵ came to the same conclusion after his minute study of the inflammatory processes involving the omentum. However, Kiyono, quoted by Cunningham, in examining omental spreads from animals vitally stained with carmine and trypan blue, could never find any indication of a transition from the lining cells to fibroblasts. He said that normally the lining cells form a continuous and characteristic cell series over the omental surfaces, and they can be easily differentiated from the fibroblasts; but in the presence of inflammation the two types may resemble each other closely. Von Büngner²⁶ came to a similar conclusion in work on granulation tissue production, as he could not detect any transition between the two groups of cells. Tschasin²⁷ later decided that he could differentiate definitely between the serosal cells and the fibroblasts by means of the vital dyes, as the former type failed to take on the stains. Cunningham²¹ noted that the fibroblasts took on more vital dyes than the serosal cells, but neither type reacted intensively. In his preparations of normal omenta which were vitally stained and silvered, the fibroblasts were seen in between the two layers of serosal cells as cells with faint outlines and finely granular inclusions in the cytoplasm. These fibroblasts were often branched, and

²² Arch. f. mikr. Anat., 1908, 72, p. 209.

²³ Compt. rend. Acad. d. sc., 1891, 112, p. 842.

²⁴ Compt. rend. Soc. de biol., 1901, 53, p. 890.

²⁵ Arch. f. mikr. Anat., 1909, 74, p. 143.

²⁶ Beitr. z. path. Anat. u. z. allg. Path., 1896, 19, p. 33.

²⁷ Folia haemat., 1913, 17, p. 317.

even though the outlines were indistinct, they could be made out because of the trypan blue in the cellular processes. In a later paper²⁸ on the reactions of the cells of the peritoneal cavity to vital dyes, he showed that the large serosal lining cells stained characteristically, as most of them contained a small ring of granules in one part of the cytoplasm. This method of storage of vital dye was considered characteristic, especially the localization of the granules in a circumscribed area in the cytoplasm and in the formation of a perinuclear rosette. It appears that the serosal cells and the fibroblasts are distinct cell types, although they appear to be closely related when subjected to external influences.

The origin of the clasmatocytes has also aroused considerable interest. Ranvier,²⁹ writing in 1899 on "Les clasmatocytes," stated that he first noticed the presence of the clasmatocytes in the femoral aponeurosis. Later he used the mesentery of the molluscs, in which he found masses of cells which resembled the ordinary leukocytes. These clasmatocytes had dendritic processes, and he considered them as derived from the fibroblasts. His further work with the dorsal lymph space of the frog and the rabbit omentum led him to conclude that the clasmatocytes were distinct from the fibroblasts. He then concluded that the clasmatocytes were derived from the leukocytes. The separation of the clasmatocyte as a distinct type of cell of the connective tissues was due to the work of Maximow.³⁰ He showed that by the introduction of two sterile cover slips under the skin of rabbits, one could separate 3 types of cells by the speed with which they passed between the covers: the leukocytes appearing first; then a special cell, the clasmatocyte, which wandered in in about 19 hours, and lastly the fibroblasts. He further showed that the clasmatocytes were specifically sensitive to neutral red. Later workers have confirmed these results and have concluded that the clasmatocyte is a cell of connective tissue origin, specifically differentiated to take up and store particulate matter. Sabin,³¹ from her careful work on the chick, concluded that the endothelium gives rise to 2 groups of cells: the megaloblasts which develop hemoglobin and become erythroblasts, and a strain of cells called histiocytes by Aschoff. The extravascular histiocytes have been termed clasmatocytes; the intravascular cells are called monocytes. She stated that the latter group is specifically differentiated for purposes of phagocytosis. Dominici³² considered the macrophages as arising from the differentiations of the various cell syncytiums which are normally present in the omentum, probably from the primitive cells. Foot³³ reached a conclusion similar to Sabin's.

Cunningham,²¹ in his omental preparations, described an additional series of cells which were elongated and loaded with trypan blue. Some of these cells were oval and found chiefly in the "taches laiteuses." He considered these typical clasmatocytes, similar to the "adventitial" cell of Marchand. Many of the cells formed in long lines along the course of the vessels, and were at times intimately connected with the endothelial cells of the capillaries. Dominici also considered the macrophages as mainly limited to the "taches laiteuses." Weidenreich²² limited the anlage of exudative cells of the peritoneal cavity to the omentum, and later Cunningham believed the clasmatocytes contained in the peritoneal exudate of the rabbit to be derived from the cellular constituents of the "taches laiteuses."

²⁸ Am. Jour. Anat., 1922, 30, p. 399.

²⁹ Arch. d'anat. micr., 1899, 3, p. 122.

³⁰ Beitr. z. path. Anat. u. z. allg. Path., 1901, 4, p. 1; Arch. f. mikr. Anat., 1906, 67, p. 680.

³¹ Bull. Johns Hopkins Hosp., 1921, 32, p. 314.

³² Arch. de méd. expér. et d'anat. path., 1902, 14, p. 1.

³³ Jour. Med. Research, 1919, 40, p. 353.

THE SITES OF ANTIBODY PRODUCTION

Ehrlich's lateral chain theory assumes that any cell that takes and binds antigen can produce the corresponding antibody or antibodies. Hektoen³⁴ states that whether the power to produce antibodies is widely distributed among cells of the body or whether it is limited more or less strictly to certain cells or tissues has not been definitely determined. Considerable effort has been spent in the attempt to determine the organs and tissues involved in the production of antibodies.

In general, the methods used have been the removal of organs which were considered as possible sites of antibody production; the use of various physical and chemical agents, generally chosen so as to have some selective action on a special tissue, and the use of different sites for the introduction of the antigens.

The hematopoietic system has been considered the most important integral part of the mechanism for the fabrication of antibodies. Certain experiments have been made to establish this assumption. Chief have been those on the effects of splenectomy and alterations in the bone marrow and lymphatic apparatus by physical and chemical agents on the subsequent course of antibody production. Hektoen³⁵ showed that splenectomy may diminish the output of antibodies, especially when practiced about the same time the antigen is introduced, but had little or no effect when the antibody production was well under way. Motohasi³⁶ came to similar conclusions, in that splenectomy in rabbits profoundly modified the production of specific hemolysin, both as to site and concentration in the various organs and in the blood stream. He also noted a compensatory hemophage activity, in splenectomized rabbits, in the bone marrow and liver after about 45 days. Hektoen had previously noted this compensatory property of the tissues concerned in antibody activity. Krumbhaar and Musser³⁷ found that splenectomized monkeys seemed to exhibit much less blood change than splenectomized dogs or men. The removal of the stomach, small intestines, and thyroid gland was found by Hektoen and Curtis³⁸ to have no effect on antibody production.

Various chemical and physical agents have been used to bring about reactions in the hematopoietic system. Thompson³⁹ was able to increase the antibody producing power of rabbits by preliminary injections of tuberculin; this reaction he attributed to the irritating effects on the endothelial phagocytes. Arkin⁴⁰ demonstrated that strychnine had a stimulating effect, while morphine, potassium cyanid and chloral had a depressing effect on phagocytosis. Sodium iodoxybenzoate has been found to stimulate lysin production in dogs, an effect which Hektoen⁴¹ and Arkin⁴² considered as probably due to acceleration of oxidation in the tissues involved in antibody production. Hektoen⁴³ also demonstrated that repeated injections of benzene in rabbits at the same time that sheep blood was injected greatly reduced the concentration of specific precipitins and lysins. The same author and Corper⁴⁴ noted that such agents as thorium X and mustard

³⁴ Jour. Infect. Dis., 1911, 9, p. 103.

³⁵ Ibid., 1920, 27, p. 23.

³⁶ Jour. Med. Res., 1922, 43, p. 419, 473.

³⁷ Arch. Int. Med., 1923, 31, p. 686.

³⁸ Jour. Infect. Dis., 1915, 17, p. 409.

³⁹ Jour. Med. Res., 1922, 43, p. 37.

⁴⁰ Jour. Infect. Dis., 1913, 13, p. 408.

⁴¹ Tr. Chicago Path. Soc., 1911, 8, p. 138.

⁴² Jour. Infect. Dis., 1915, 16, p. 349.

⁴³ Ibid., 1916, 19, p. 69.

⁴⁴ Ibid., 1920, 26 p. 320; 1921, 28, p. 279.

gas had a similar restraining effect on antibody elaboration. In the experiments of the latter type the depressant action of the chemical agents on antibody formation is associated with a marked decrease in the circulating leukocytes and with destruction of the leukocyte precursors in the bone marrow.

The roentgen ray and radioactive substances have profound effects on the lymphatic and blood forming apparatus. Nakahara⁴⁵ has shown that there is a marked stimulation of the lymphoid tissue subsequent to small doses of radiation. Murphy and Morton⁴⁶ had similar results. Russ and his co-workers⁴⁷ found that large doses of radiation caused a marked decrease in the circulating lymphocytes. Hektoen⁴⁸ demonstrated that roentgen-ray exposure of rats before immunization reduced the lysin formation, whereas this agent had no definite effect after the process was well under way. He had similar results with the simple exposure of dogs and also when splenectomy was combined with the radiation. Likewise, radium emanation given intravenously in sublethal doses had a depressing effect on lysin production (Hektoen and Corper⁴⁹). Ivy and his co-workers⁵⁰ do not believe that the effects of radiation on the spleen and lymph glands are due to stimulation but rather to irritation.

Zinsser⁵¹ stated that the tissue cell, as the functional unit, must be looked on as the source of the various protective constituents of normal and immune serum. Kyes⁵² and later Cary⁵³ found that the organs with great hemophage activity, in the destruction of foreign red blood corpuscles, have a higher concentration of specific antibody. Kyes postulated the theory that the hemophages are the producers of antibodies specific for cells they take up. Cantacuzene,⁵⁴ in trying to locate the formation of precipitins, found that the spleen, bone marrow and lymph nodes contained precipitins in increasing amount up to the seventh day of immunization and on that day the precipitins appeared in the blood. Also that extracts of leukocytes, obtained in the pleural exudates resulting from aleuronat injections, contained precipitins earlier than the blood. Since mononuclear cells predominate in the experimental exudate, and precipitins are found first and in the highest concentration in the organs rich in mononuclears, he concluded that these cells probably form the precipitins. Krause and Schiffman⁵⁵ were unable to demonstrate the presence of precipitins in the extracts of various organs of the rabbits although the serum contained this antibody.

Many writers have attempted to prove that tissues, other than those forming the hematopoietic system, may be included in the antibody formers. Römer⁵⁶ found that abrin injected into the conjunctival sac caused the local formation of antitoxin at a stage corresponding to antitoxin production called forth by abrin, which is absorbed into the general circulation. Hektoen⁵⁷ after injection of antigen into the anterior chamber of dogs was unable to find any evidence of antibody in the aqueous humor until after newly formed antibodies appeared in

⁴⁵ Jour. Exper. Med., 1919, 24, p. 83.

⁴⁶ Ibid., 1915, 22, p. 800.

⁴⁷ Lancet, 1919, 1, p. 692.

⁴⁸ Jour. Infect. Dis., 1915, 17, p. 415; 1918, 22, p. 28; 1920, 27, p. 23.

⁴⁹ Ibid., 1922, 31, p. 304.

⁵⁰ Jour. Radiol., 1923, 4, p. 189.

⁵¹ Infection and Resistance, 1918, p. 200.

⁵² Jour. Infect. Dis., 1916, 18, p. 277.

⁵³ Jour. Med. Res., 1922, 43, p. 399.

⁵⁴ Ann. de l'Inst. Pasteur, 1908, 22, p. 54.

⁵⁵ Ibid., 1906, 20, p. 225.

⁵⁶ Arch. f. Opht. 1901, 52, p. 72.

the blood. Wassermann and Citron³⁷ injected typhoid bacilli intravenously, intraperitoneally, and intrapleurally into dogs. They then injected 5 c.c. of aleuronat on the 9th day into the pleural cavity and 10 c.c. of normal salt solution intraperitoneally. The dogs were bled the next day and the pleural and peritoneal exudates withdrawn. Their results indicated the possibility of the local formation of antibody by the cells of the peritoneum and pleura. Hektoen,³⁴ however, found that in most cases intrapleural injection of the antigen gave less concentration of antibody in the serum than intravenous injection, and in no case after intrapleural injection of the antigen did the antibody content in the pleural exudate exceed that of the serum. Klauder and Kolmer³⁸ found that the local Wassermann reaction was of much more value than the blood test in primary syphilis, and that it yielded as high a percentage of positive reactions as the dark field method. Russell³⁹ claimed that the subcutaneous injection of typhoid vaccine in typhoid fever is beneficial. He attempted to explain his results by the inherent power of the connective tissue to form antibodies, and supposed that this type of tissue in the subcutaneous region does not normally react in the usual course of the disease. Hektoen³⁴ was unable to confirm the increased production of antibodies after multiple subcutaneous injections of the antigen. The recent work of Gay and Morrison⁴⁰ has shown that successful resistance to streptococcus pleurisy is constantly associated with a persistently high clasmatocyte count. They consider that this local form of immunity to streptococcus is associated with the "tissue macrophages" or clasmatocytes.

The experimental work reviewed points to a widespread distribution in the body of the function of forming soluble antibodies. But it has not succeeded in proving that antibody formation is a specific property of any particular tissue or cell type. It indicates that those groups of tissues and cell species which make up the hematopoietic system are most active, but it has not as yet definitely determined which of the cell constituents of this system are chiefly concerned in the elaboration of antibodies; nor has it shown to what degree this function may reside in certain cell types, situated outside the hematopoietic tissues, which are common to the latter and the other tissues of the body.

EXPERIMENTS

Rabbits, guinea-pigs and dogs were used. The operative procedures were carried out under ether anesthesia. The omentum was removed after careful ligation of the larger arteries and veins as they leave the gastroepiploic vessels along the greater curvature of the stomach. Care was taken not to interfere with the splenic artery as it passes through the upper border of the gastrosplenic omentum. The animals were

³⁷ Ztschr. f. Hyg. u. Infektionskrankh., 1905, 50, p. 331.

³⁸ Jour. Am. Med. Assn., 1921, 76, p. 1635.

³⁹ Boston Med. & Surg. Jour., 1911, 164, p. 1.

⁴⁰ Jour. Am. Med. Assn., 1923, 80, p. 1298; Jour. Infect. Dis., 1923, 33, p. 338.

allowed to recuperate for a period of two weeks, after which time the antigen was introduced into the peritoneal cavity through a small incision in the abdominal wall. They were then bled every 2 days. Healthy normal animals were used as controls.

TABLE 1
INTRAPERITONEAL IMMUNIZATION OF RABBITS WITH DEFIBRINATED SHEEP BLOOD

Days	Rabbit 2			Rabbit 4			Control Rabbit 1		
	Lysins	Precip- itin	Agglu- tinins	Lysins	Precip- itin	Agglu- tinins	Lysins	Precip- itin	Agglu- tinins
2	24	10	0	12	10	0	6	160	0
5	96	80	0	384	40	12	1,536	640	96
7	384	320	24	1,536	640	96	3,072	2,560	192
9	768	640	48	3,072	2,560	192	12,288	5,120	768
12	3,072	1,280	96	6,144	2,560	384	24,576	5,120	1,536
14	6,144	1,280	96	6,144	2,560	192	24,576	5,120	768
16	3,072	640	96	6,144	2,560	96	12,288	2,560	384
19	1,536	640	96	3,072	1,280	48	6,144	1,280	192
21	1,536	320	48	3,072	1,280	48	6,144	1,280	96
23	1,536	320	24	1,536	1,280	48	3,072	1,280	48
25	1,536	320	24	768	1,280	24	3,072	1,280	96
28	768	160	24	768	640	24	3,072	640	96
30	384	160	24	384	640	24	1,536	640	48
34	384	80	24	384	320	12	768	320	24
37	192	40	12	384	320	6	768	320	24
41	192	20	12	192	80	6	384	80	24
44	192	20	6	192	80	6	384	80	12
50	96	10	6	96	40	6	192	80	12
60	96	10	3	96	20	6	192	40	6
70	0	0	0	48	10	0	96	10	6

TABLE 2
INTRAPERITONEAL IMMUNIZATION OF DOGS WITH DEFIBRINATED SHEEP BLOOD

Days	Dog 2		Dog 3		Control Dog 1		Control Dog 2	
	Lysins	Agglu- tinins	Lysins	Agglu- tinins	Lysins	Agglu- tinins	Lysins	Agglu- tinins
1	0	0	0	0	0	0	0	0
3	12	12	12	6	12	12	24	6
5	768	48	384	48	768	96	3,072	48
7	6,144	192	3,072	96	6,144	384	6,144	192
9	24,576	384	12,288	192	24,576	768	12,288	384
12	12,288	192	6,144	96	12,288	384	12,288	192
14	12,288	192	6,144	96	6,144	192	6,144	96
16	6,144	96	3,072	48	3,072	96	3,072	48
18	3,072	96	1,536	48	1,536	96	3,072	48
20	1,536	96	768	48	768	48	3,072	24
23	768	48	768	24	192	48	1,536	24
24	384	48	384	12	96	24	768	12
28	192	24	192	6	96	12	192	6
30	96	12	48	6	48	6	96	6
40	96	6	24	0	48	6	96	6
50	48	6	12	0	48	0	48	0
60	24	0	6	0	24	0	48	0
70	24	0	6	0	12	0	24	0

The serum was separated from the blood, heated at 56 C. for 30 minutes, and then the antibody concentration determined. For lysin content, mixtures were made of 0.2 c c. of a 5% suspension of washed sheep or chicken corpuscles in normal salt solution; 0.003 c c. of guinea-

pig serum as complement (0.006 c.c. was necessary for the chicken hemolytic system); and dilutions of the rabbit, dog or guinea-pig serum to be tested in normal salt solution. The total volume in each case was 0.6 c.c. This mixture was incubated at 37 C. for 2 hours and then placed in the icebox over night. Next morning the highest dilution of the serum causing hemolysis was recorded. For the precipitin concentration, the highest dilution of sheep or chicken serum in which the rabbit, dog or guinea-pig serum caused a definite precipitate, by the contact method, after standing at room temperature for 1 hour, was recorded. The agglutinin titers were determined by means of mixtures of 0.2 c.c. of a 5% suspension of washed sheep or chicken corpuscles and dilutions of inactivated rabbit, dog or guinea-pig serum, in salt solution, the total volume in each case being 0.6 c.c. These mixtures were incubated for 2 hours and then placed in the icebox over night, and next morning the highest dilution of each serum causing distinct agglutination of the corpuscles was noted.

Removal of the Omentum and Intraperitoneal Immunization.—The omentum was removed from a series of rabbits as described. Two weeks later, under light anesthesia, a small incision was made through the anterior abdominal wall and 15 c.c. of defibrinated sheep blood were introduced. Several controls were immunized in a similar manner at the same time. The rabbits were bled from the marginal ear vein every 2 days, and the concentration of the antibodies in the serum determined by the methods indicated above. The results are given in table 1 and chart 1. The experiments were repeated several times with similar results.

Two weeks after removing the omentum in a number of dogs, 25 c.c. of defibrinated sheep blood were introduced intraperitoneally. Several controls were immunized at the same time in a similar manner. The dogs were bled every two days and the concentration of the antibodies determined (table 2 and chart 2).

Similar experiments were made in guinea-pigs, using 5 c.c. of defibrinated sheep blood. These animals were bled from the heart at frequent intervals and the antibody content determined (table 3).

As seen in tables 1-3, and charts 1 and 2, the general course of the antibody curves corresponds to that of the typical curve following a single injection of antigen described by Hektoen and Carlson.⁶¹ The control rabbits produced about 4 times the quantity of antibodies at the height of the curve as did the rabbits deprived of the omentum, and

⁶¹ Jour. Infect. Dis., 1910, 7, p. 319.

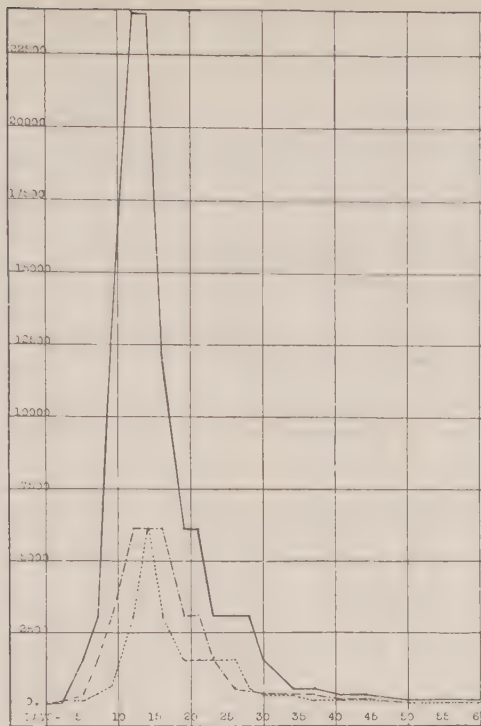


Fig. 1.—Lysins produced in rabbits following intraperitoneal injection of sheep blood. The dash and dot line indicates the omentum removed; the dotted line, the omentum removed; the continuous line, the omentum intact.

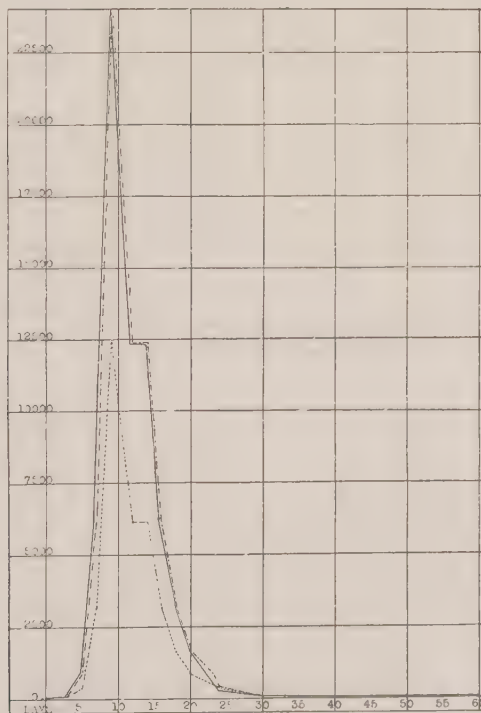


Fig. 2.—Lysins produced in dogs following intraperitoneal injection of sheep blood. The dash and dot line indicates the omentum removed; the dotted line, the omentum removed; the continuous line, the omentum intact.

TABLE 3
INTRAPERITONEAL IMMUNIZATION OF GUINEA-PIGS WITH DEFIBRINATED SHEEP BLOOD

Days	Guinea-Pig 3			Control Pig 3			Control Pig 4		
	Lysins	Precip- itin	Agglu- tinins	Lysins	Precip- itin	Agglu- tinins	Lysins	Precip- itin	Agglu- tinins
3	0	0	0	0	0	0	0	0	0
6	0	0	6	0	0	6	0	0	6
9	6	0	12	0	0	12	6	0	12
12	12	0	24	6	0	12	12	0	24
14	96	0	48	12	0	12	96	0	48
16	48	0	24	6	0	12	48	0	24
18	24	0	24	0	0	6	24	0	12
21	6	0	12	0	0	0	12	0	6

TABLE 4
INTRAVENOUS IMMUNIZATION OF RABBITS WITH DEFIBRINATED SHEEP BLOOD

Days	Rabbit 19			Rabbit 20			Control Rabbit 20		
	Lysins	Precip- itin	Agglu- tinins	Lysins	Precip- itin	Agglu- tinins	Lysins	Precip- itin	Agglu- tinins
1	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	24	10	6
5	96	20	6	96	20	6	384	160	24
7	768	160	48	768	160	48	1,536	320	48
9	1,536	640	96	1,536	320	96	1,536	640	96
11	1,536	1,280	96	1,536	640	96	1,536	1,280	96
13	768	640	48	768	320	48	1,536	640	48
15	768	640	24	384	160	24	768	320	48
17	384	320	12	384	160	12	768	320	24
19	192	160	6	192	80	6	384	160	12
21	96	80	6	96	40	0	192	80	12
23	96	40	0	48	40	0	96	80	6
25	48	10	0	24	10	0	96	40	6
27	24	10	0	12	0	0	48	10	6
30	12	10	0	6	0	0	24	10	0

TABLE 5
INTRAVENOUS IMMUNIZATION OF DOGS WITH DEFIBRINATED SHEEP BLOOD

Days	Dog 4		Dog 5		Control Dog 3	
	Lysins	Agglutinins	Lysins	Agglutinins	Lysins	Agglutinins
2	0	0	0	0	0	0
4	6	0	6	6	6	0
6	192	48	192	48	96	24
9	384	48	384	96	192	48
11	768	96	768	96	768	96
13	1,536	192	1,536	192	1,536	192
16	768	96	1,536	192	768	96
18	768	48	768	96	384	48
20	384	24	384	48	192	24
23	192	12	192	48	96	12
25	192	6	96	24	96	6
27	96	6	48	12	48	6
30	48	6	24	6	12	6

the active antibody production continued longer in the control rabbits. In dogs and guinea-pigs, however, there was no marked difference in the concentration of antibodies produced by the animals with and without the omentum.

Removal of the Omentum and Intravenous Immunization.—Two weeks after removing the omentum from a number of rabbits and dogs, 2 c.c. of defibrinated sheep blood were injected into the ear vein of the rabbits and the external jugular vein of the dogs. At the same time control animals were injected in the same way. The animals were subsequently bled every 2 days and the antibody content of the serum determined. The results in the rabbits are recorded in table 4 and those of the dogs in table 5.

TABLE 6
INTRAPERITONEAL IMMUNIZATION OF RABBITS WITH DEFIBRINATED SHEEP BLOOD AND
REMOVAL OF THE OMENTUM DURING THE COURSE OF ANTIBODY PRODUCTION

Days	Rabbit 21			Rabbit 22			Rabbit 23		
	Lysins	Precip- itin	Agglu- tinins	Lysins	Precip- itin	Agglu- tinins	Lysins	Precip- itin	Agglu- tinins
Normal	0	0	0	0	0	0	0	0	0
2	96	0	6	96	0	6	192	0	6
4	192	10	12	192	10	12	384	10	12
4	Omentum removed			80 Omentum removed			1,280 Omentum removed		
8									
8	384	20	24	768	80	48	1,536	40	96
10	1,536	320	48	768	160	48	3,072	640	192
12	3,072	640	96	3,072	640	96	6,144	1,280	192
12	Omentum removed			1,280 Omentum removed			2,560 Omentum removed		
14									
14	3,072	640	96	6,144	1,280	192	12,288	2,560	384
17			...	3,072	640	96	6,144	640	192
20			...	1,536	320	48	3,072	320	96

As seen in tables 4 and 5, the rabbits deprived of the omentum produced about an equal concentration of antibodies in the serum as the control animals, after intravenous injection of the antigen. The dogs also reacted in a similar manner.

Sheep serum was then used as a soluble antigen and the previous experimental work repeated. Similar results were obtained as with sheep blood. Chicken blood was substituted for the sheep blood, and like results were noted.

Removal of the Omentum at Varying Stages of Antibody Production.—In another series of rabbits, 5 c.c. of defibrinated sheep blood were injected intraperitoneally, and the omentum was removed on the 4th, 8th and 12th days. They were bled every 2 days, and the concentration of antibodies in the serum was determined (table 6).

Removal of the omentum during the process of antibody production had a definite influence on the subsequent antibody course. This was most marked when the omentum was removed at the end of 4 days, and less marked after 8 days.

Removal of the Omentum and Spleen with Intraperitoneal Immunization.—In other rabbits and dogs, 2 weeks after removing the omentum, the spleen was removed and defibrinated sheep blood introduced into the peritoneal cavity, 15 c.c. in each rabbit, and 25 c.c. in each of the dogs. At the same time, animals from which only the omentum had been removed, as well as suitable controls, were immunized in a similar manner. These animals were bled at frequent intervals and the antibody content determined (table 7).

TABLE 7
REMOVAL OF THE OMENTUM AND SPLEEN IN RABBITS WITH INTRAPERITONEAL
IMMUNIZATION WITH DEFIBRINATED SHEEP BLOOD

Days	Rabbit 12. Spleen and Omentum Removed			Control Rabbit 10 Omentum Removed			Control Rabbit 11 No Operations		
	Lysins	Precip- itin	Agglu- tinins	Lysins	Precip- itin	Agglu- tinins	Lysins	Precip- itin	Agglu- tinins
2	48	20	12	48	10	6	96	26	12
4	96	40	24	192	80	24	384	160	48
6	768	320	48	768	320	48	1,536	640	96
7	1,536	640	192	3,072	640	384	6,144	2,560	768
9	3,072	640	384	6,144	1,280	768	12,288	2,560	768
11	6,144	1,280	768	12,288	2,560	1,536	24,576	5,120	1,536
13	6,144	1,280	768	6,144	2,560	768	24,576	5,120	1,536
15	3,072	1,280	384	6,144	1,280	384	12,288	2,560	768
16	1,536	640	192	3,072	640	192	6,144	1,280	384
18	1,536	640	192	3,072	640	192	3,072	1,280	384
21	768	320	96	1,536	320	96	1,536	640	192
23	384	320	48	768	320	96	768	640	96
25	384	320	48	768	320	48	768	640	96
30	384	160	48	768	160	48	768	320	96

It was found, first, that splenectomy alone produced a definite decrease in antibody production. When splenectomy followed the removal of the omentum there was a more marked decrease than after simple removal of the omentum.

Omental Transplants and Their Influence on Antibody Production.—The omentum was removed from several rabbits and dogs and, in 2 weeks omentum from normal animals was transplanted into the peritoneal cavity of the experimental animals. In a few, the omentum was transplanted in one piece spread over the abdominal viscera. In others, the omentum was cut into small pieces of about 1 sq. cm., and inserted in various places. The animals were killed at the end of two weeks,

and the omental transplants studied histologically. It was found that with both methods of transplantation the omentum soon became converted into acellular fibrous masses which were adherent to the peritoneum.

In similar series, defibrinated sheep blood was injected at the time of omental transplantation, and the subsequent course of antibodies noted (table 8).

TABLE 8
TRANSPLANTED OMENTUM AND INTRAPERITONEAL IMMUNIZATION OF RABBITS WITH
DEFIBRINATED SHEEP BLOOD

Days	Rabbit 17 Transplanted Omentum			Rabbit 18 No Omentum			Control Rabbit 19 Normal		
	Lysins	Precip- itin	Agglu- tinins	Lysins	Precip- itin	Agglu- tinins	Lysins	Precip- itin	Agglu- tinins
1	0	0	0	0	0	0	12	0	0
2	0	0	0	6	0	0	48	10	6
4	6	0	6	24	10	6	192	20	12
6	192	80	12	384	160	12	768	320	48
8	768	320	24	1,536	640	48	3,072	1,280	96
10	1,536	640	24	3,072	640	48	6,144	2,560	192
12	3,072	1,280	96	6,144	1,280	196	12,288	5,120	768
14	3,072	1,280	48	6,144	1,280	192	24,576	5,120	768
16	1,536	640	24	3,072	1,280	96	12,288	2,560	384
18	1,536	640	24	1,536	640	48	6,144	1,280	192
20	768	320	12	768	320	24	3,072	640	96
22	768	160	12	384	160	12	1,536	320	96
24	768	160	12	384	160	12	1,536	320	96
26	384	80	6	192	80	12	1,536	320	96
28	384	80	6	192	80	6	1,536	320	48
30	192	40	6	192	80	6	768	160	24

As indicated in table 8, rabbits with transplanted omentum did not produce more antibodies than rabbits deprived of the omentum, both giving less than the normal controls.

MORPHOLOGY OF THE OMENTUM

The excised omentum was fixed in a 3% solution of potassium bichromate in 10% formalin for 24 hours and then washed in distilled water for 12 hours. Portions of the thinner parts were used for permanent preparations. Two methods of staining were found most suitable:

1. The usual hematoxylin-eosin stain.
2. Grenacher's alum carmine method, which consisted in staining the tissue 24 hours in 2% carmine in a 5% potassium alum solution. The tissue was then washed in distilled water until it was free from the excess of stain and was then immersed in a saturated alcoholic picric acid solution for 5 minutes, dehydrated, cleared in creosote and finally

mounted as a stretched preparation in balsam. The latter method stained the nuclei red, so that it could be used as a good counterstain for vital staining with trypan blue.

Rabbit Omentum.—The omenta removed from all the experimental rabbits showed moderate variations in gross and microscopic appearances. There were, however, marked differences in size according to the age. The young rabbits had a thin, small, membranous structure, whereas the mature animals had a well defined and in some cases extensive membrane, which frequently measured 13 by 7 cm. The tissue was



Fig. 3.—Sketch of the rabbit omentum showing the characteristic gross appearance of this structure with the branching blood vessels and numerous "taches laiteuses."

transparent except at the insertion along the greater curvature of the stomach, and the splenic and duodenal regions, where it was somewhat fibrous and contained adipose tissue. The vessels formed a treelike structure, with small cellular areas scattered along them. These "taches laiteuses" were equally numerous throughout the membrane, and many had no demonstrable vascular connections.

Specimens, fixed and stained according to the methods given, revealed a uniform and peculiar structure (fig. 3). There were two definite mesothelial layers, between which were the fibroblasts, clasmatocytes, and vessels. The "taches laiteuses" had a characteristic struc-

ture (fig. 4). Those that occurred along the blood vessels had the appearance of a glomerulus, formed by interlacing capillary tufts with well developed afferent and efferent vessels; however, there were many cellular islands which have no demonstrable vascular connections. In addition to the vascular structures of the large islands, there were many cells scattered through them, some of which were elongated and oval, while others were more round and had smaller nuclei. Many of the

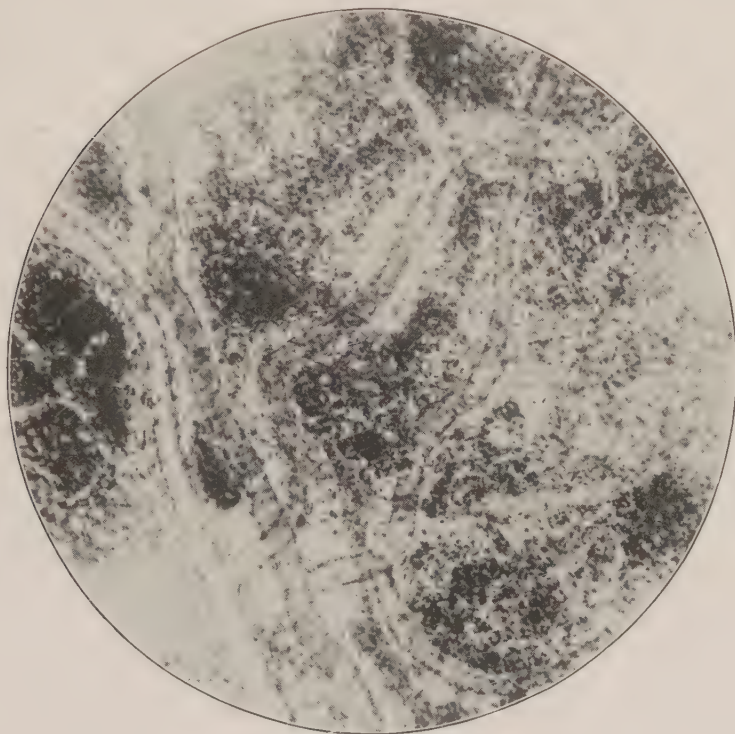


Fig. 4.—Low power representation of a vascular "tache laiteuse" of a rabbit omentum, showing the interlacing capillary tufts and extensive cellular accumulations. $\times 85$.

cells had long branching intercellular processes. These different cell types were brought out clearly by the use of vital stains, to be described.

In order to observe the reaction of the rabbit omentum to granular material introduced into the peritoneal cavity, 10 c.c. of India ink were injected intraperitoneally. The omentum was removed at the end of 24 hours, fixed and stained with hematoxylin and eosin. It was found that the omentum was jet black, and in some places the ink formed a

thick coating. On microscopic study the ink was found contained chiefly within large phagocytic cells, most numerous in the "taches laiteuses." Similar results were obtained when a 5% suspension of carmine was substituted.

Vital staining of the rabbit omentum was found most satisfactory by the use of 4 subcutaneous injections of 2 c.c. of 1% trypan blue at daily intervals. The dye appeared in the general circulation after a period of 15 minutes, stained the serum a deep blue after one hour, and caused a generalized stain at the end of 24 hours. The preparations revealed a characteristic distribution of the dye. The serosal lining cells took on little stain, the fibroblasts more, and the clasmatocytes most, the latter sometimes being loaded with the blue granules. The fibroblasts were usually branched, and the blue granules could be traced into the various prolongations. The clasmatocytes were somewhat elongated or oval in shape and were found chiefly in the "taches laiteuses."

Experiments on the method of phagocytosis by the omentum were made next. One consisted in the introduction of 20 c.c. of a mixture, containing 10 c.c. of a 5% suspension of carmine in 25% acacia in normal salt solution and 10 c.c. of defibrinated chicken blood, into the peritoneal cavity of several rabbits. The omentum was removed at the end of 24 hours, fixed and stained in the usual manner. It was found that the corpuscles and carmine granules were taken up in large quantities by the phagocytic cells found chiefly in the "taches laiteuses." Some of the clasmatocytes contained from 8 to 12 nuclei of the chicken corpuscles. The cell bodies of the erythrocytes became indistinct, although there were many "ghosts" left within the cytoplasm of the phagocytes. The second procedure utilized rabbits which had been vitally stained with trypan blue and then received a suspension of chicken corpuscles in acacia mixture. The omentum removed at the end of 24 hours showed a picture similar to that obtained with the first method, but the clasmatocytes contained both blue granules and numerous chicken nuclei.

Since the rabbit omentum takes up particles from the peritoneal cavity, it was decided to study this process at frequent intervals after the introduction of chicken blood. Several rabbits were vitally stained with trypan blue. Then 10 c.c. of a mixture containing 5 c.c. of defibrinated chicken blood and 5 c.c. of a 25% acacia suspension were injected into each peritoneal cavity. The omentum was removed at the end of 12,

24, 48 and 96 hours, was fixed and stained in the usual manner. This study showed that at the end of 12 hours there was practically no fluid in the peritoneal cavity, but that many smaller and larger clumps were adherent to the omentum. The stained preparations showed many chicken red cells adherent to the external surface of the omentum, and a few intact erythrocytes contained within the vitally stained phagocytic

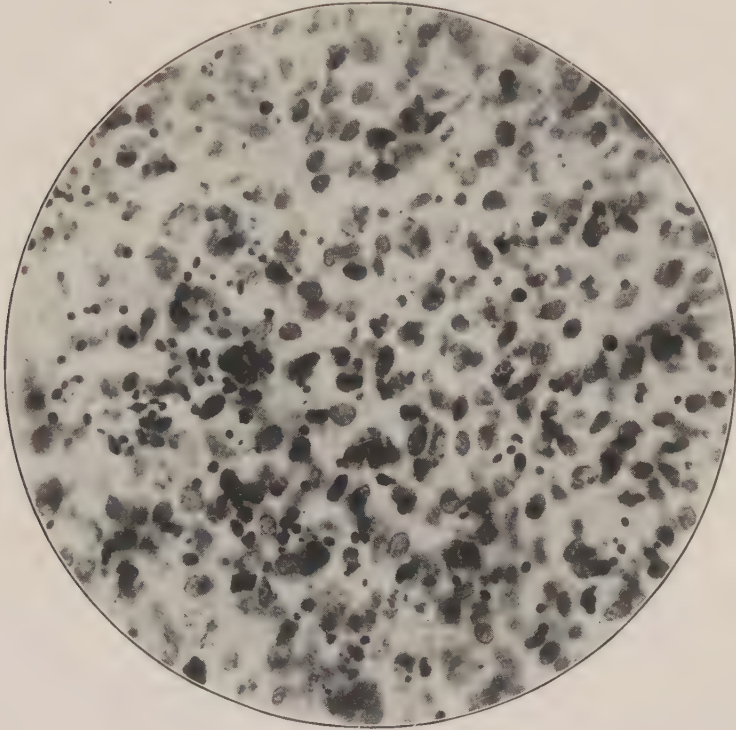


Fig. 5.—Higher magnification of the cells in an omentum removed 24 hours after injection of chicken blood, illustrating the method and degree of phagocytosis by the clasmotocytes. Many of the chicken corpuscles are not as yet completely taken up and are seen as small dark nuclei surrounded by an indistinct rim of cytoplasm. $\times 350$.

cells in the "taches laiteuses." However, at the end of 24 hours these cells were loaded with the nuclei from the chicken corpuscles, while the cell bodies could no longer be recognized (fig. 5). After 48 hours there was considerable fragmentation of these nuclei, so that they were almost unrecognizable. At the last stage, there was considerable granular material contained within the cytoplasm of the cells.

The same experimental method was carried out on another group of vitally stained rabbits, except that in this set the rabbits had received 5 c.c. of defibrinated chicken blood 12 days previous to the second injection of 10 c.c. of a mixture containing 5 c.c. of defibrinated chicken corpuscles and 5 c.c. of a 25% acacia suspension. The omentum was removed at the end of 6, 12 and 24 hours. After 6 hours, about 7 c.c.

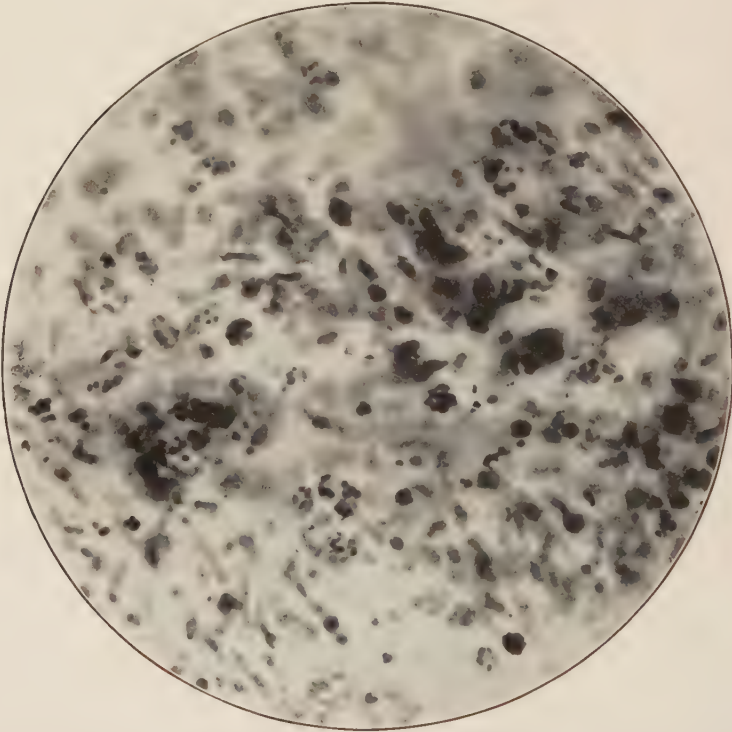


Fig. 6.—Similar to fig. 5, except that the omentum was removed at the end of 6 hours from a rabbit which was at the height of immunity. This structure exhibits a more advanced and complete degree of phagocytosis although observed 18 hours earlier than the nonimmunized rabbit. $\times 350$.

of fluid deeply stained with laked blood were found free in the peritoneal cavity. This exudate contained "ghosts" of corpuscles, swollen and edematous nuclei, and an occasional intact red cell. The preparations of the omentum showed the clasmotocytes loaded with the chicken corpuscle nuclei and containing numerous blue granules (fig. 6). The animals studied after 12 and 24 hours contained a similar amount of

blood tinged fluid, only there were no red cells and few intact nuclei in the smears of the exudate. The omentum showed a more advanced degree of phagocytosis and earlier fragmentation of the nuclei than that seen in the nonimmunized animals. Intraperitoneal hemolysis was present. It was also noted that the clasmatocytes in these preparations contained more trypan blue and seemed more numerous than in the rabbits which had received only one injection of the blood.

In order to determine the relation of this increased vital staining to functional activity, 10 c.c. of defibrinated sheep blood were injected into the peritoneal cavity of several rabbits, and omentum removed on the 4th, 8th, and 12 days. Each animal received 4 daily injections of trypan blue prior to removing the omentum. This structure was fixed and stained in the usual manner. This study showed that the omentum, at the end of 4 days, took on more stain than the controls, and the "taches laiteuses" were more prominent. At the end of 8 and 12 days, there was an increased number of clasmatocytes, increased vascularity of the cellular islands and more intense vital staining of the clasmatocytes. It would appear that the ability of the macrophages to take on trypan blue might be used as an index of functional activity. Similar experiments were carried out with antigen injected intravenously. The omentum removed at varying stages of antibody production showed a slight increase in the number of clasmatocytes in the "taches laiteuses" with some increase in the vital staining. The same type of experiments were made with sheep serum, and similar results were obtained.

Dog Omentum.—The omentum of the dog is an extensive, apron-like membrane (fig. 7). The main structure consists of a dense fibrous network containing a large amount of adipose tissue and large blood vessels. The membranous part between these supporting tissues is semitransparent. The vessels form extensive anastomoses throughout, but there is a conspicuous absence of the cellular islands seen in the rabbits. There are small cellular aggregations of phagocytes along the vessels. The omentum was studied at the height of antibody production, but there were no definite discernible reactions.

Guinea-Pig Omentum.—The omenta from many guinea-pigs revealed a rather uniform picture (fig. 8). This structure measured about 8 by 3.5 cm., was a thin membrane, except along the insertion to the greater curvature of the stomach. The groundwork consisted of interlacing intercellular processes surrounding vacuoles of varying size. The nuclei of the cells were oval or round, although some were

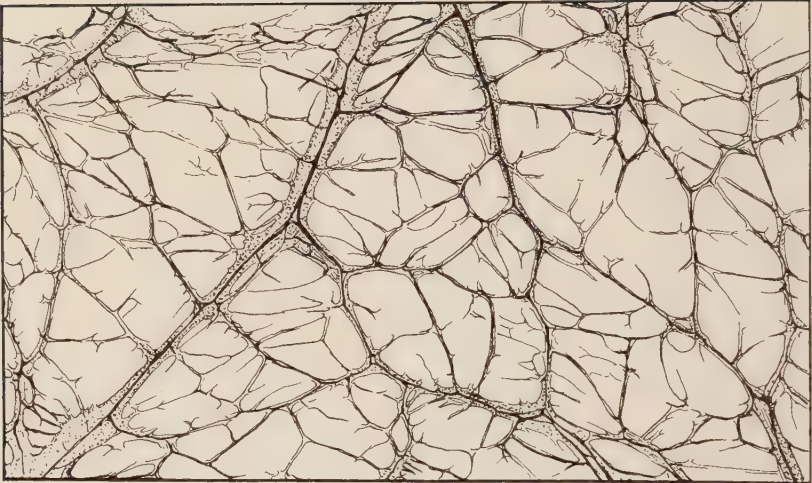


Fig. 7.—Sketch of the dog omentum, showing the characteristic gross appearance of this membrane with its vascular framework and absence of the phagocytic cells.



Fig. 8.—Sketch of the guinea-pig omentum, demonstrating its general structure with the numerous glomerulus-like bodies scattered along the blood vessels.

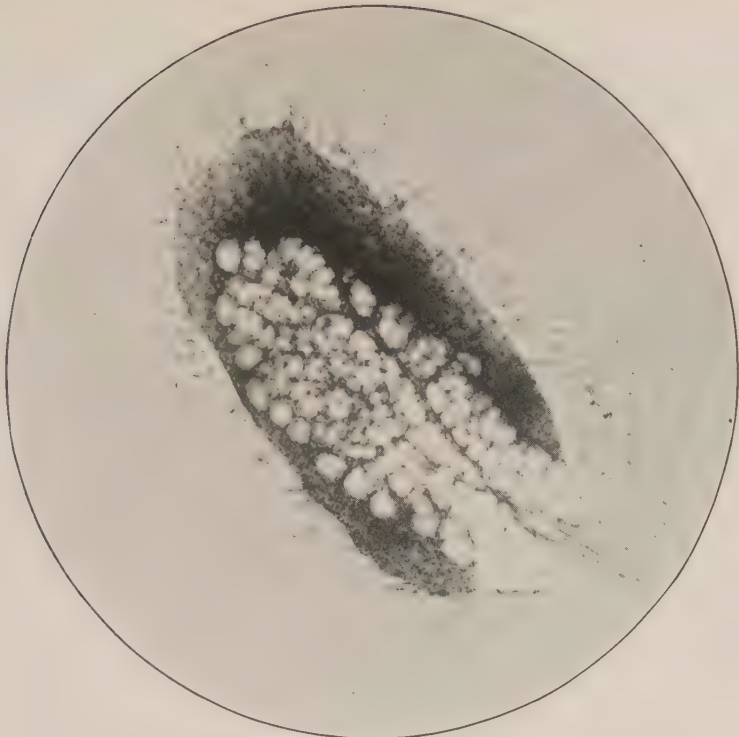


Fig. 9.—Low power representation of a glomerulus-like body in guinea-pig omentum, showing the characteristic central capillary network and the peripheral cellular aggregations. $\times 60$.

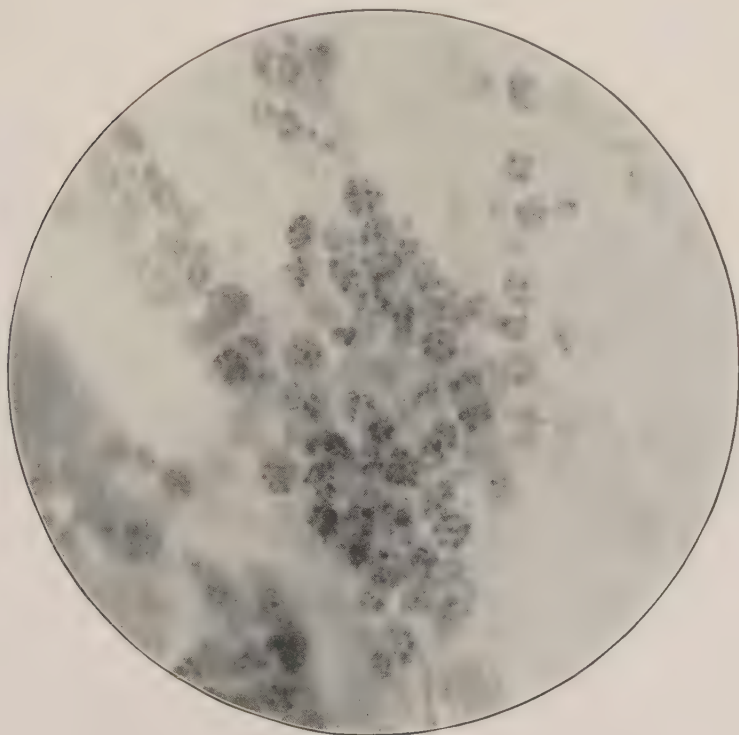


Fig. 10.—Higher power representation of the marked phagocytosis of chicken corpuscles by the cellular constituents of the glomerulus-like bodies of the guinea-pig omentum removed after 24 hours. $\times 350$.

elongated and spindle shaped. The larger vessels had a fibro-fatty supporting tissue, in which were embedded many small accumulations of phagocytic cells. Many of the terminal vessels ended in a glomerulus-like structure supplied by one or two small arterioles and drained by one vein. The peripheral part of these bodies was usually made up of cellular aggregations (fig. 9).

Trypan blue was injected into several guinea-pigs. The omentum was removed, fixed and stained. It was found that the cells took out only a small amount of stain from the general circulation, and this was well confined to the cells composing the glomerulus-like bodies. Chicken corpuscles were injected into the peritoneal cavities of several vitally stained guinea-pigs. The omentum was removed at the end of 24 hours. There was only a moderate degree of phagocytosis and this chiefly by the phagocytic cells described (fig. 10).

A group of experiments was made similar to those performed in the rabbit. The results obtained corresponded with the lessened ability of the guinea-pig to produce antibodies. The degree of phagocytosis of the immunized animals was somewhat increased over that of the normal, but there was an entire absence of the intraperitoneal hemolysis which occurred in the immunized rabbits. The phagocytic cells seemed to take on only a slightly increased amount of trypan blue at the height of antibody production.

DISCUSSION

The early experiments demonstrated that rabbits deprived of their omentum produced, at the height of the antibody curve, about one-fourth the concentration of antibodies in their serum, in response to a single intraperitoneal injection of antigens, of that produced by normal control rabbits. Removal of the omentum soon after the introduction of the antigen also caused a similar decline, but this had little effect when practiced later than the eighth day after the onset of immunization. Rabbits without the omentum did not show any decrease in antibody production after intravenous injection of the antigens. Sheep blood and serum, and chicken blood were used as the antigens. The results were similar with both the cellular and soluble antigens but not as marked with the latter. The antibody production in dogs and guinea-pigs was independent of the presence of the omentum following both intraperitoneal and intravenous injection of the antigens. In rabbits, splenectomy when combined with the removal of the omentum produced a more decided decrease in the subsequent concentration than

simple removal of either organ. Omental transplants did not remain viable and had no influence on the antibody production in animals which received this tissue at the time of injection of the antigen.

It was found advisable, because of this discrepancy resulting from intraperitoneal and intravenous injections of the antigens in rabbits and of the species differences, to make a careful morphologic study of the various omenta, and as stated it was found that the omentum in the three groups has an entirely different structure. The rabbit has a well developed organ with numerous "taches laiteuses," while the omentum of the guinea-pig with its glomerulus-like bodies stands next, and finally comes that of the dog, which is nearly devoid of well defined phagocytic cellular islands. The vascularity of the three also shows various gradations; the dog being most vascular, the rabbit next, and the guinea-pig least. These gross differences were further emphasized by detailed study of the individual cell types in their reactions to foreign material and blood introduced into the peritoneal cavity, and to vital stains.

The rabbit omentum readily takes up granular and cellular materials from the peritoneal cavity. The latter reaction is shown clearly by the use of chicken corpuscles, which because of their nuclei can be distinguished with ease. The most characteristic feature, however, is that the most marked phagocytosis occurs in the cells comprising the "taches laiteuses." It was noted further that this process is greatly accelerated by previous immunization. The injected foreign blood also becomes laked in the peritoneal cavity of the immunized rabbit, a phenomenon not observed in the control rabbits. The guinea-pig omentum, likewise, takes up the chicken corpuscles chiefly in the cells which are situated about the periphery of the glomerulus-like bodies. This phagocytic activity is not much increased by previous immunization, and no intraperitoneal hemolysis of the foreign corpuscles ensues. The omentum of the dog exhibits little phagocytosis, and this property is not augmented by previous immunization.

Cunningham has demonstrated that the three cell types of the rabbit omentum can be differentiated by injections of trypan blue as a vital stain. My omental preparations confirm these results, as the cells of the serosal lining take on little dye and have a pavement-like arrangement forming two distinct layers. The fibroblasts are elongated branched cells, scattered throughout the membrane but most numerous along the blood vessels and in the region of "taches laiteuses." These

cells contain much more trypan blue than the serosal lining cells, and frequently contain granules extending out into intercellular processes. The clasmatocytes react in a characteristic manner. They are found chiefly in the cellular islands, frequently filled with blue dye and either oval or round, with a somewhat vesiculated nucleus. These cells are the chief phagocytic elements of the omentum and often have from 8 to 10 nuclei of chicken erythrocytes 48 hours after injecting the blood. The clasmatocytes at the height of immunity show a more intense degree of vital staining and phagocytize the corpuscles more readily and completely. Similar cells are also found scattered throughout the membrane. This increase in vital staining of the clasmatocytes becomes progressively more intense between the initial injection of the antigen and the height of the antibody curve, which occurs about the 12th to 14th day. The guinea-pig omentum contains similar phagocytic cells about the periphery of the glomerulus-like bodies. This cell type also takes on vital stain the most intensively of the cellular constituents of the structure, but does not demonstrate a definitely increased staining capacity during the course of antibody production.

The relationship between phagocytosis and antibody production could not be definitely established. The rabbit with its highly developed phagocytic activity produced a high concentration of antibodies, and when deprived of the omentum with its numerous clasmatocytes, had its antibody function restricted. The dog with little similar phagocytic reaction produced antibodies comparable to those of the rabbit, but did not produce the same variety of antibodies in response to the same antigen. The results also showed that removal of the omentum from the dog with its few phagocytic cells has no lowering effect on the subsequent course of antibody production. The differences in the responses in the species may be a quantitative variation in the number of local clasmatocytes stimulated at the site of injection of the antigen and possibly a qualitative difference, as precipitins are not formed in the dog in response to injections of sheep blood. The early morphologic study of the guinea-pig omentum seemed to indicate that this structure should be between that of the rabbit and the dog, as there is a well defined phagocytic activity in the structures similar to the "taches laiteuses" of the rabbit omentum. However, the guinea-pig produces little soluble antibodies, and these in a low concentration. The difference between the guinea-pig and the rabbit is evidently a qualitative one. The differences between the guinea-pig and the dog are quantitative

as to the number of the phagocytic cells and qualitative as to the variation of antibody reaction to the same antigen. The clasmatoocytes of the rabbit omentum seem to be a definite factor in antibody production in this species, because the removal of the omentum reduces the antibody-producing ability of the rabbits after intraperitoneal injection of the antigen; because of the increased phagocytosis by these cells at the height of immunity; and because of the occurrence of intraperitoneal hemolysis of foreign corpuscles in immunized animals.

Cunningham states that the variation in the storage of vital dyes in the cells represents corresponding differences in physiologic adaptation. The increased vital staining of the clasmatoocytes at the height of antibody production probably indicates, then, a greater functional activity of these cells, possibly in producing antibodies.

SUMMARY

The omentum of the rabbit takes part in the development of antibodies in response to intraperitoneal injection of antigen, but in the dog and guinea-pig the omentum seems far less active in this respect.

The differences are probably due to variations in the structure of the omentum of the three species. The rabbit omentum has well defined "taches laiteuses" consisting chiefly of clasmatoocytes, that of the dog has only few cellular islands, while the guinea-pig omentum contains glomerulus-like bodies, but this animal has little power to produce free antibodies.

The omentum of the rabbit shows greater activity at the height of antibody production, as evidenced by increased phagocytic ability and more intense degree of vital staining of the clasmatoocytes, and intraperitoneal lysis of foreign corpuscles.

Transplanted rabbit omentum soon is converted into a fibrous tissue devoid of endothelial cells.

The clasmatoocytes of the rabbit omentum appear to be a definite factor in antibody production following intraperitoneal injection of antigen.

